Cationic phosphorous dendrimers as complexing agents for siRNA delivery

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ABSTRACT SUMMARY

Cationic phosphorous-containing dendrimers were investigated for complexation with siRNA and their physicochemical characteristics were evaluated. Dendriplexes were successfully formed for all three generation of dendrimers with their size ranging between 80-180nm depending on the generation. siRNA was tightly bound to the dendriplexes and the dendrimers showed reasonably low cytotoxicity.

INTRODUCTION

Dendrimers have been widely investigated as non-viral vectors for gene and drug delivery systems. They possess several functional surface groups which are responsible for their high solubility and ability to carry a high charge density [1]. Poly(amidoamine) (PAMAM) dendrimers are the class of dendrimers most often used as vehicles for nucleic acid delivery and have repeatedly shown good transfection efficiency and biocompatibility [2].

In this study, different cationic phosphorous dendrimers were studied for complexation with a model siRNA directed against luciferase. Three different generations of the dendrimers, Gen 1, Gen 2 and Gen 3 were tested at different amine-to-phosphate (N/P) ratios (5, 10, 20 and 40), charge ratio between cationic dendrimer (N) and anionic siRNA (P).

The purpose of this study was to characterize the formation of dendriplexes between siRNA and dendrimers, with respect to their size, morphology, binding and toxicity.

EXPERIMENTAL METHODS

The dendrimers were synthesized by the Laboratoire de Chimie de Coordination du CNRS as previously published by Loup et al [3]. Dendriplexes were prepared by adding the dendrimer solution to the siRNA solution (both in 10mM Hepes buffered saline at different dendrimer-to-siRNA molecular ratios) and vortexing the solution.

The particle size distribution and polydispersity index (PDI) of the dendriplexes were determined by dynamic light scattering. Samples were prepared in triplicates and three measurements were performed for each sample using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK).

CryoTEM images were taken using a Tecnai G2 20 (FEI, Oregon, USA). Samples were prepared by placing them onto carbon-coated grids, blotting with filter paper and vitrifying the sample in liquid ethane. Samples were scanned at an accelerating voltage of 200kV.

Gel electrophoresis was performed using ethidium bromide, dendriplexes were loaded in the agarose wells with methylene blue and siRNA bands were visualized using a Kodak Image Station 1000 (Kodak, Rochester, NY, USA). Pure siRNA was used as control.

Cell toxicity studies were performed on A549 lung cells using an MTT assay. 96-well plates were seeded with 8000 cells per well and dendriplexes were added up to a concentration of 30μg/ml using N/P ratio 10. Cell viability was measured 24 hours after incubation with dendriplexes using a fluorescence plate reader.

RESULTS AND DISCUSSION

The size of dendriplexes were measured at four different N/P ratios (5, 10, 20 and 40) with a net positive charge. Size measurements showed dendriplexes between 80-190nm (Fig 1). It is observed that the dendriplexes increase in size with increasing dendrimer generation. The size was further dependent on different mixing parameters. The polydispersity indices
(PDI) of the dendriplexes are shown in Table 1 and indicate that the PDI increased with increasing dendrimer generation at all N/P ratios. Similar studies with PAMAM dendrimers showed an opposite trend, with dendriplexes becoming smaller with increasing dendrimer generation [4]. A cryoTEM image of dendriplexes prepared with generation 1 dendrimers at N/P ratio of 1 (Fig 2) confirmed the approximate size of the dendriplexes and showed spherical complexes.

Gel electrophoresis (Fig 3) showed that for all dendrimers siRNA was trapped in the wells tightly bound to the dendriplexes and free unbound siRNA was not detected. The brighter bands for higher NP ratios are explained by the stronger cationic charge of the dendriplexes.

Table 1. Polydispersity indices of dendriplex aggregates prepared at different NP ratios and different dendrimer generations.

<table>
<thead>
<tr>
<th>N/P ratio</th>
<th>Gen 1</th>
<th>Gen 2</th>
<th>Gen3</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.17</td>
<td>0.30</td>
<td>0.39</td>
</tr>
<tr>
<td>10</td>
<td>0.18</td>
<td>0.35</td>
<td>0.35</td>
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<tr>
<td>20</td>
<td>0.21</td>
<td>0.27</td>
<td>0.36</td>
</tr>
<tr>
<td>40</td>
<td>0.19</td>
<td>0.27</td>
<td>0.36</td>
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The cytotoxicity results showed 50-60% cell viability at 10 μg/ml for Gen 2 and 3 dendrimers and 100% cell viability for Gen 1 dendrimers, while at 3 μg/ml 100% cell viability was observed for all three generations. The phosphorous dendrimers thus had some toxicity towards these cells at high concentrations.

Figure 1. Size of dendriplex aggregates prepared at different N/P ratios and using dendrimers of different generations. Measurements were made in triplicates and error bars indicate the standard deviation.

CONCLUSIONS

siRNA-loaded dendriplexes were prepared from cationic phosphorous dendrimers of three different generations and it was demonstrated that the dendrimers were capable of producing complexes of suitable size with good binding affinity for prospective delivery of siRNA.

REFERENCES


ACKNOWLEDGMENTS

The authors would like to thank the Danish Agency for Science, Technology and Innovation for financial support of this work.